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Stack Test

Protocol

E. I. du Pont de Nemours & Company
Fayetteville Works
Fayetteville, North Carolina

9 February 2006

SECTION 1

INTRODUCTION

The E. I. Du Pont Fayetteville Works operates a process that could have the potential to emit ammonium perfluorooctanoate (APFO) via an exhaust stack. APFO is used as a wetting agent in the manufacture of some Teflon products. The plant has elected to voluntarily undertake a program to determine the emission rate of APFO by conducting a stack test.

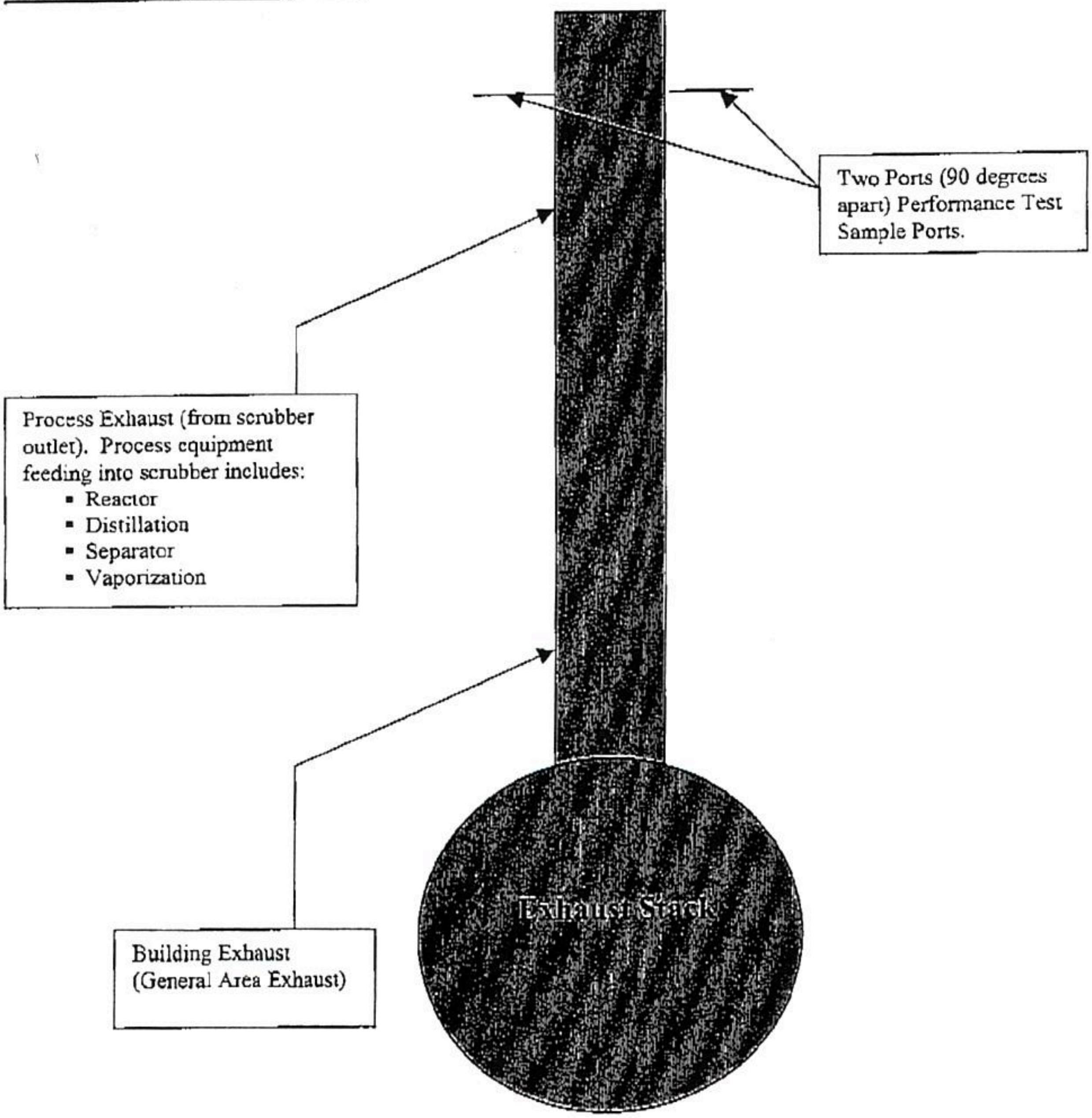
Testing will be conducted for a "Virgin" Campaign batch and a "Purification" Campaign batch with sampling times of approximately 30 hours and 24 hours, respectively. A sample will be collected approximately once five hours during the Virgin Campaign batch and once every four hours during the Purification Campaign batch. Thus, it is expected that a total of six sampes will be collected for each batch.

This protocol will specify the test methods and techniques that are believed best for capture of APFO from a gas matrix. The remainder of this document will provide guidance on the preferred methods to accomplishing the test objectives. It is divided into the following sections:

Section 2	Source Identification
Section 3	Sampling Test Method Selection
Section 4	Method 5 Modifications
Section 5	Sample Preparation
Section 6	Analytical Method

SECTION 2

SOURCE IDENTIFICATION



SECTION 3

TEST METHOD SELECTION

The methods to be used during the test program are summarized in Table 1. The cited methods are referenced and documented in 40 CFR Part 60 Appendix A per the most recent update of the CFR. The only exceptions to the cited methods occur in the ***Reference Method 5--Determination of Particulate Matter Emissions from Stationary Sources.*** Section 4 provides description of each modification and its justification. Section 5 will describe the cleaning of the sample bottle and the sample storage.

The data from the test program will be summarized in appropriate tables in a final report along with all applicable calibration data. Standard conditions are chosen to be **20° C (68° F)** and **1 Atm (29.92 in. Hg)** unless requested otherwise by the regulatory agency.

Table 1
Emission Test Method Summary

PARAMETER	METHOD
Stack sampling layout	EPA Reference Method 1--Sample and Velocity Traverses for Stationary Sources
Gas velocity and volumetric flow rate	EPA Reference Method 2--Determination of Stack Gas Velocity and Volumetric Flow Rate (Type S Pitot Tube)
Gas Molecular Weight	EPA Reference Method 3--Gas Analysis for the Determination of Dry Molecular Weight
Moisture	EPA Reference Method 4--Determination of Moisture Content in Stack Gases
APFO (Particulate and Vapor Phases)	EPA Reference Method 5--Determination of Particulate Matter Emissions from Stationary Sources (Modified)
Sample Analysis	Per Analytical Method performed by off-site laboratory (See Section 6).

SECTION 4

METHOD 5 MODIFICATIONS

The following modifications to Reference Method 5 are being recommended to improve the collection of APFO and enhance its detectability. These modifications do not in any way invalidate the basic sampling technique of the method. Unless otherwise specified all other requirements of the method are to be followed.

Modification	Justification
Probe/box temperature set at no more than 10° F above stack temperature	Stack gas temperature is ambient.
Replace plain distilled water with ASTM Type 1 Reagent Grade water in the impingers.	This combination will provide the best solution for absorbing the surfactant.
Five (5) impinger train configuration	The modification is intended to improve the collection of APFO by providing an additional absorption zone. The impingers will be loaded as follows: • Impingers1, 2 and 3 filled with 100-ml each of distilled water solution. • Impinger 4 is empty. • Impinger 5 contains desiccant.
Sample recovery	Sample recovery will be performed with water to remain consistent with the sample collection reagent. All samples will be properly labeled and submitted to an offsite lab for analysis (See Section 6).

SETION 5

SAMPLE PREPARATION

The samples are to be stored in prewashed high density polyethylene bottles. The prewash protocol for bottles and glassware will consist of washing the bottle in 0.1N sulfuric acid, rinsing in distilled/deionized water, followed by air drying in a dust free enclosure. ***At no time will soaps or detergents be used in the process as they may contain the target analyte which is a surfactant.*** The bottles will be placed in zip lock plastic bags for transport and remain sealed until needed. Labels may be affixed to the bottles prior to their placement in the sealed in the zip lock plastic bag.

The sample filters will be placed in a Petri dish, which will be put into a Ziploc-type bag, and then labeled and sealed. Pre- and post-weighing of the filters is not required since the analyte will be determined chemically..

All liquid samples will be stored in a precleaned plastic bottle. The volume as well as all necessary information will be noted on the bottle. Each bottle will be tape closed to prevent leaking.

A field blanks consisting of 500-ml of the water solution and one filter will be reserved and shipped with the field samples.

SECTION 6

ANALYTICAL METHOD

Performed by off-site analytical company. See the attached document for details on analytical methodology. If there are any questions on the methodology, please contact Mike Johnson at the DuPont Fayetteville Works site.

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SECTION 6

PREFERRED ANALYTICAL METHOD

The analysis of the field samples will be done by a Dupont approved laboratory using previously validate and accepted methodology. However, for throroughness, an overview of the analytical protocol is included for both the aqueous samples and the sample filter.

- **Impginer Water Analysis [Appendix A]**
  Method Title: Method of Analysis for the Determination of Perfluorooctanioc Acid (PFOA) in Water, Rev. 2, Exygen Research, State College, PA 16801, Method No. 01M-008-046 Revision 2 (V0001013), 15 August 2005.

- **Filter Analysis [Appendix B]**
  Method Title: Method of Analysis for the Determination of Perfluorooctanioc Acid (PFOA) in Filter Paper by LC/MS/MS Rev. 1, Exygen Research, State College, PA 16801, Method No. V0001920 Rev. 1, 15 August 2005.

APPENDIX A

Method of Analysis for the Determination of Perfluorooctanioc Acid (PFOA) in Water
Revision 2

TABLE OF CONTENTS

LIST OF FIGURES

Reason for revision: Added changes as result of discussions between DuPont and EPA.

1. SUMMARY

This report details a method of analysis for residues of ammonium perfluorooctanoate (APFO) in water.

APFO forms perfluorooctanoic acid (PFOA) in solution. PFOA is extracted from water using C_{18} solid phase extraction (SPE) cartridges. Quantification of PFOA is accomplished by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis using selected reaction monitoring (SRM). Residues of PFOA found are mathematically converted and reported as APFO. The chemical formula of APFO is given in Section 2 of this method.

The anticipated limit of quantitation (LOQ; the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) for this method is 50 ng/L (parts-per-trillion) [or 0.05 micrograms/liter] for APFO.

Quantification is performed using calibration standards prepared in filtered HPLC water and processed through the extraction procedure, identical to samples. During the extraction procedure, all samples (including standards, controls, fortifications etc.) are concentrated by a factor of eight; therefore, the smallest standard (25 ng/L) injected during the chromatographic run is equivalent to 200 ng/L (concentrated 8×). The method detection limit (MDL) is approximately 6 to 15 ng/L depending on the instrument used for analysis. This value is based on procedures found in 40 CFR Part 136 Appendix B.

This method was developed using stream water, well water, tap water, and drinking water (bottled water). Typical percent recoveries ± standard deviations (at 100 and 500 ng/L) for APFO in tap water were 112% ± 4.1%; in well water were 104% ± 2.3%; in stream water were 108% ± 3.3%; and in drinking water were 110% ± 3.3%.

A representative calibration curve is shown in **Figure 1.** Representative chromatograms are shown in **Figures 2 to 15.**

3.3. EQUIPMENT AND SUPPLIES

Equipment	Supplier
Balance, analytical (display at least 0.0001 g)	Mettler
Vacuum pump	Buchi
Visiprep vacuum manifold	Supelco
Sep Pak Vac 6 cc (1g) tC18 cartridges (part # WAT 036795)	Waters
50 mL disposable polypropylene centrifuge tubes	VWR
15 mL disposable polypropylene centrifuge tubes	VWR
Disposable glass micropipets (50-100 µL, 100-200 µL)	Drummond (VWR)
25 mm diameter glass fibre acrodisc 0.45 µ (cat #4523)	Gelman
Class A pipets and volumetric flasks	various suppliers
Hypercarb drop-in guard column (4 mm) (part # 844017-400)	Keystone
Stand-alone drop-in guard cartridge holder	Keystone
125-mL LDPE narrow-mouth bottles	Nalgene
HPLC pump (LC10AD)	Shimadzu
2 mL clear HPLC vial kit (cat # 5181-3400)	HP
Standard lab equipment (graduated cylinders, disposable tubes etc.)	various suppliers
LC/MS/MS and HPLC systems	As described in section 4.5.

Note: Equivalent materials may be substituted for those specified in this method if they can be shown to produce satisfactory results.

Notes:

1. In order to avoid contamination, the use of disposable labware is highly recommended (tubes, pipets, etc.).
2. PTFE or PTFE lined containers or equipment, including PTFE-lined HPLC vials for the HPLC autosampler must not be used.
3. HPLC grade water used during the sample and standard extraction should be filtered through a Hypercarb guard column using a HPLC pump, as described in section 4.2. This water is referred to as "filtered HPLC water", here after in this report.
4. It is necessary to check the solvents (methanol) for the presence of contaminants by LC/MS/MS before use. Certain lot numbers have been found to be unsuitable for use.
5. Use disposable micropipets or pipets to aliquot standard solutions to make calibration standards and sample fortifications.

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3.4. SOLUTIONS

(1) 50 mM ammonium acetate solution is prepared by weighing 3.86 g of ammonium acetate and dissolving in 1 L of HPLC water. Dilute the 50 mM solution by a factor of 25 to make the 2 mM ammonium acetate solution used for mobile phase A.

(2) 40% methanol is made by measuring 400 mL methanol and adjusting the volume to 1 L with filtered HPLC water.

(3) 250 mg/mL sodium thiosulfate solution is made by dissolving 25 g in 100 mL filtered HPLC water.

Note: The aforementioned examples are provided for guidance, alternative volumes may be prepared as long as the ratios of the solvent to solute ratios are maintained.

3.5. PREPARATION OF STANDARDS AND FORTIFICATION SOLUTIONS

Analytical standards are used for three purposes:

1. Calibration Standards – These standards are prepared in filtered HPLC water and are used to calibrate the response of the detector used in the analysis.
2. Laboratory Control Spikes – These fortifications are prepared at concentrations corresponding to the LOQ and 10x LOQ and are used to determine analytical recovery. Laboratory control spikes are prepared in filtered HPLC water.
3. Matrix Spikes – These fortifications are prepared by spiking into the field samples at concentrations ranging from 500 ng/L up to 500,000 ng/L. Matrix spikes are used to evaluate the effect of the sample matrix on analytical recovery.

The absolute volumes of the standards may be varied by the analyst as long as the correct proportions of solute to solvent are maintained.

3.5.1. Stock solution

Prepare a stock solution of 100 µg/mL of PFOA by weighing out 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 100-mL volumetric flask. The stock solution (in 125-mL LDPE bottles) is to be stored in a refrigerator at 2°C to 6°C and is stable for a maximum period of 6 months from the date of preparation.

3.5.2. Fortification Solutions

a. Prepare a fortification standard of 1.0 µg/mL (1000 ng/mL) of PFOA by adding 1.0 mL of the 100 µg/mL stock solution to a 100-mL volumetric flask and bring up to volume with methanol.
b. Prepare a fortification standard of 0.1 µg/mL (100 ng/mL) by diluting 10.0 mL of the 1.0 µg/mL solution to 100 mL with methanol in a volumetric flask.
c. Prepare a fortification standard of 0.01 µg/mL (10 ng/mL) by diluting 10.0 mL of the 0.1 µg/mL solution to 100 mL with methanol in a volumetric flask.

Example: two hundred microliters of the 10 ng/mL solution spiked into 40 mL of water is equivalent to a 0.05 ppb (50 ng/L) fortification.

Store all fortification standard solutions in a refrigerator (in 125-mL LDPE bottles) at 2°C to 6°C for a maximum period of 3 months from the date of preparation, after which time it is necessary to make new standards using the stock solution. Note also that additional concentrations may be prepared if necessary.

3.5.3. Calibration Standards

LC/MS/MS calibration standards are prepared in HPLC water filtered through a hypercarb drop-in guard cartridge. The calibration standards are processed through the extraction procedure, identical to samples. The extracted standards may be used for a period of two weeks when stored refrigerated (at 2°C to 6°C).

The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Fortification Volume (µL)	Volume of Fortified Control Sample (mL)	Final Concentration of Calibration Standard (ng/L)*	Calibration Standard ID (example)
0	0	40	0	Xcmmddyy-0
10	100	40	25	Xcmmddyy-1
10	200	40	50	Xcmmddyy-2
10	400	40	100	Xcmmddyy-3
100	100	40	250	Xcmmddyy-4
100	200	40	500	Xcmmddyy-5
100	400	40	1000	Xcmmddyy-6

* The extracted concentration of the calibration standard is equal to 8x its initial concentration, due to the concentration of the standard during the extraction (SPE). XC = extracted calibration standard.

A zero standard solution (reagent blank) <u>must be prepared</u> with each set of standards extracted. This standard is used only to access the reagents

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used to prepare the standards and is <u>not</u> included as part of the calibration curve.

Store all extracted calibration standards in 15-mL polypropylene tubes at 2°C to 6°C, up to two weeks.

3.5.4 Controlling Standard Bias

Prepare a separate stock solution of 100 µg/mL from a different PFOA neat standard from a different source by weighing out 10 mg of analytical standard (corrected for purity only) and dilute to 100 mL with methanol in a 100-mL volumetric flask. The stock solution (in 125-mL LDPE bottles) is to be stored in a refrigerator at 2°C to 6°C and is stable for a maximum period of 6 months from the date of preparation.

Prepare a fortification standard of 1.0 µg/mL (1000 ng/mL) of PFOA by adding 1.0 mL of the 100 µg/mL stock solution to a 100-mL volumetric flask and bring up to volume with methanol. From that solution, prepare a fortification standard of 0.1 µg/mL (100 ng/mL) by diluting 10.0 mL of the 1.0 µg/mL solution to 100 mL with methanol in a volumetric flask.

Prepare a 250 ng/L extracted calibration standard with each set of extracted standards. Add 100 µL of the 100 ng/mL fortification solution to 40 mL of hypercarb filtered HPLC water and process through the extraction procedure, identical to samples.

> Note: This standard is referred to as the "check standard" from this point forward.

The check standard should be included at least once with each set of extracts analyzed. The check standard should give a response within ± 15% of the average response from the 250 ng/L extracted calibration standards in the set.

4. METHOD

4.1. FLOW DIAGRAM

The flow diagram of the method is given below, followed by a detailed description of each step.

Method Flow Diagram

Measure 40 mL of sample
(fortify samples designated as calibration standards and fresh fortifications)
↓
↓
C_{18} Solid-Phase Extraction
↓
↓
Final Volume (5 mL)
↓
↓
LC/MS/MS

4.2. SAMPLE PROCESSING

No sample processing is needed for water samples. However, frozen samples must be allowed to completely thaw, un-aided, at room temperature. Samples stored refrigerated should also be allowed to equilibrate to room temperature. All samples must be thoroughly mixed and any visible solids removed by filtering the sample through a 25-mm diameter glass fibre acrodisc 0.45 µ filter before being sampled for extraction.

Initial studies show that the method is not effected by the pH of the sample. Sample pH is recorded prior to extraction.

Control HPLC water, used for the preparation of samples and calibration standards as well as water used for equilibrating and washing the SPE cartridges, must be prepared by filtering HPLC water through a hypercarb guard cartridge using a HPLC pump. Before use, wash the guard cartridge with ~ 25 ml HPLC grade acetonitrile, then ~25 mL of HPLC grade methanol, followed by ~ 25 mL of HPLC water using the HPLC pump at ~ 2 mL/min flow rate. Then start collecting the filtered HPLC water eluate from the cartridge. It is recommended that the cartridge be washed, as described above, after filtering ~ 2 liters of HPLC water. A flow rate of 2 to 3 mL/min is recommended.

4.3. SAMPLE PREPARATION

a. Each batch of samples extracted (typically 20 or less) must include at least one reagent control (method blank using filtered HPLC water) and two reagent controls fortified at known concentrations to verify procedural recovery for the batch.

b. Each sample must be extracted in replicate unless the sample is marked as a field blank. Field blanks do not need to be extracted in replicate.

c. Each sample extracted (except for field blanks) must also be separately fortified at a known concentration and carried through the procedure to verify recovery. Matrix spikes should range from 500 ng/L to 500,000 ng/L. If the sample residue is found to be significantly higher than the level at which the sample was initially fortified, the sample should be fortified at a higher concentration (up to 500,000 ng/L) and re-extracted.

4.4. EXTRACTION

1. Measure 40 mL of sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).
   <u>*Note:*</u> *For tap water samples and any water samples suspected to contain chlorine, add 200 µL of a 250 mg/mL sodium thiosulfate solution to the 40 mL sample and mix well to de-activate chlorine, before fortification.*
2. Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL filtered HPLC water (~ 2 drop/sec). Do not let column run dry.
   <u>*Note:*</u> *For the following steps, maintain a ~1 drop/ sec flow rate. Do not allow the column to run dry at any time.*
3. Load sample on conditioned C_{18} SPE cartridge. Discard eluate.
4. Wash with ~5 mL 40% methanol in water. Discard eluate.
5. Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).
6. Analyze samples using electrospray LC/MS/MS.

NOTE: SAMPLES ARE CONCENTRATED BY A FACTOR OF EIGHT DURING THE EXTRACTION; INITIAL VOL = 40 ML → FINAL VOL. = 5 ML.

4.5. QUANTITATION

4.5.1. LC/MS/MS System and Operating Conditions (Electrospray)

Mass Spec:	Micromass Quattro Ultima (Micromass)
Interface:	Electrospray (Micromass)
	Harvard infusion pump (Harvard Instruments), for tuning
Computer:	COMPAQ Professional Workstation AP200
Software:	Windows NT, MassLynx 3.3
HPLC:	Hewlett Packard (HP) Series 1100
	HP Quat Pump
	HP Vacuum Degasser
	HP Autosampler
	HP Column Oven

Note: A 4 × 10 mm hypercarb drop-in guard cartridge (Keystone, part # 844017-400) is attached on-line after the purge valve and before the sample injector port to trap any residue contaminants that may be in the mobile phase and/or HPLC system.

HPLC Column:	Genesis C_8 (Jones Chromatography), 2.1 mm x 50 mm, 4μ
Column Temperature:	35° C
Injection Volume:	15 μL
Mobile Phase (A):	2 mM Ammonium Acetate in HPLC water
Mobile Phase (B):	Methanol

Time	% A	% B	Flow Rate (mL/min)
0.0	60	40	0.3
0.4	60	40	0.3
1.0	10	90	0.3
7.0	10	90	0.3
7.5	0	100	0.3
9.0	0	100	0.4
9.5	60	40	0.4
13.5	60	40	0.4
14.0	60	40	0.3
15.0	60	40	0.3

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance. Columns with different dimensions (e.g. 2.1 x 30) and also columns from different manufacturers (Keystone Betasil C_{18} etc.) could be used, provided equivalent chromatography is obtained.

Ions monitored:

Analyte	Mode	Transition Monitored	Approximate Retention Time
PFOA	Negative	413 → 369	~5 min.

The retention times may vary, on a day to day basis, depending on the batch of mobile phase etc. Drift in retention times (up to ±2 %) is acceptable within an analytical run, as long as the drift continues through the entire analysis and the standards are included at the beginning and end of the analytical run.

Note: An alternative LC/MS/MS system may be used once demonstrated to be equivalent.

4.5.2. Example MS Operating Parameters

The following MS operating values are provided as an example only. Actual values will most likely vary from instrument to instrument. These values may also change over time even when analyzing samples on the same instrument. The values are changed to optimize for greatest sensitivity. For example, hexapole 1 voltage may be set to 2 V; however, as time goes on, hexapole 1 voltage may be increased or decreased. The exact values used each time a set is analyzed will be documented with each data set.

The mass spectrometer is tuned for the analyte by infusing a ~ 0.2 µg/mL standard solution of PFOA (at 10 µL/min, using an infusion pump) via a "T" into a stream of mobile phase containing 40% methanol and 60% 2mM ammonium acetate at 0.2 mL/min flow rate. The analyte is initially tuned for the parent ion and then tuned for the product ion. Once the instrument is tuned, the optimized parameters are saved as a tune file. This tune file is then used during routine analysis.

Analyte	Dwell (s)	Collision Energy (eV)	Cone (V)
PFOA	0.2	10	10

Source	Set
Capillary	3.0 kV
Hexapole 1	0 V
Aperture 1	0 V
Hexapole 2	0 V
Source Block Temp.	100°C
Desolvation Temp.	300°C

Analyzer	Set
LM Res 1	14.0 V
HM Res 1	14.0 V
IEnergy 1	1 V
Entrance	-2 V
Exit	2 V
LM Res 2	14.0 V
HM Res 2	14.0 V
IEnergy 2	2.0 V
Multiplier	700 V

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Gas Flows	Set
Cone Gas	~ 130 L/hr
Desolvation	~ 750 L/hr
Pressures	**Read back**
Gas Cell	~ 3.0e-3 mbar

4.5.3. Calibration Curve Procedures

a. Inject the same aliquot (between 10 to 25 μL) of each extracted calibration standard processed in matrix (ranging from the lowest level standard to the highest level prepared), into the LC/MS/MS (the final concentrations of these calibration standards will be equivalent to 8x the concentration of the initial standard due to the concentration during SPE).

b. Use linear standard curves for quantitation. The zero calibration standard is not included in the calculation of the calibration curve. Linear standard curves are generated for each analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system. Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that may be excluded must not exceed 20% of the total number of extracted standards injected.

c. The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

A typical calibration curve for PFOA can be found in Figure 1.

4.5.4. Sample Analysis

a. Inject the same aliquot (between 10 to 25 μL) of each standard, sample, recovery, control, etc. into the LC/MS/MS system.

b. Standards corresponding to at least five or more concentration levels (starting with the LOQ level or below) must be included in an analytical set (note that the calibration standards are prepared by spiking filtered HPLC water and processing similar to actual samples).

c. An entire set of extracted calibration standards should be injected at the beginning of a set followed by extracted calibration standards interspersed approximately every 5-10 samples (to account for a second set of extracted standards). As an alternative, an entire set of extracted calibration standards may be included at the beginning and at the end of a sample set. In either case, extracted calibration standards must be the first and last injection in a sample set.

d. Inject the check standard once with each set of extracts.

e. The concentration of each sample/fortification/control is determined from the standard curve, based on the peak area of each analyte. The standard responses should bracket responses of the residue found in each sample set. If necessary, dilute the samples to give a response within the standard curve range.

f. Fortification recoveries falling within 70 to 130% are considered acceptable.

g. The total holding time between sample collection and analysis must not exceed 14 days. Extracted samples must be stored refrigerated between 2°C to 6°C until analysis.

h. Field samples in which either no peaks or peaks less than the MDL are detected at the corresponding analyte retention time will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention time that are less than the LOQ and greater than or equal to the MDL will be reported as NQ (not quantifiable). All chromatograms are considered raw data and as such, will be included in the data package with each report.

The analysis performed during the method development included fortifications at 100 and 500 ng/L of PFOA in well water, tap water, and stream water (collected locally, State College, PA) and bottled drinking water (from a local grocery store).

4.6. Acceptance Criteria

The following criteria must be met to ensure the presence of PFOA:

1. Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide. This transition would not discriminate between linear and branched forms of PFOA; therefore, both, if present, would be included in the calculation. If the branched isomer peaks are resolved or partially resolved from the linear peak, the summed peak areas will be used in the calculation.

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2. Method blanks must not contain PFOA at levels greater than the LOD. If a blank contains PFOA at levels greater than LOD, then a new blank sample must be obtained and the entire set must be re-extracted.

3. Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. If a matrix spike being used to evaluate an entire extraction set falls outside 70-130%, the entire set must be re-extracted. If a matrix spike being used to evaluate an individual sample falls outside 70-130%, the matrix spike along with its corresponding sample and replicate must be re-extracted.

4. Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.

5. The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

6. The response from the check standard should be within ± 15% of the average response from the 250 ng/L calibration standard concentrations. If the standard response falls outside these limits, appropriate steps must be taken to adjust instrument operation and the set of samples should be reanalyzed. If the standard response is still outside the ± 15% then standard preparations should be reviewed, and if warranted, new stock, fortification and extracted calibration solutions should be prepared. Both sets of data would then be included in the data package.

7. Retention times between standards and samples must not drift more than ± 2 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

4.7. Mass Calibration Criteria

In order to verify mass resolution, a calibration check is performed weekly on the mass spectrometer using a NaI/Rb/Cs tuning solution. The tuning solution is infused onto the mass spec using an infusion pump set to a flow rate of 10 µL/min. An MS scan from 80 to 1380 amu is performed on both MS1 and MS2. The calibration is considered acceptable if the following 11 peaks are found within ± 0.2 amu of their known masses on both MS1 and MS2: 84.9, 132.9, 172.8, 322.7, 472.6, 622.5, 772.4, 922.3, 1072.2, 1222.1 and 1372.0. If any of the masses are outside the ± 0.2 amu, then the instrument will be recalibrated and another calibration check performed.

4.8. Performance Criteria

The following two criteria must be performed as a system suitability test, before the commencement of analysis when using an instrumentation set-up that has not been used for this method.

First Criterion:
Run a standard solution on LC/MS/MS corresponding to the estimated LOQ (50 ng/L) in matrix and obtain a signal to noise ratio of the 413 → 369 transition of at least 9:1, compared to a reagent blank. If this criterion cannot be met, optimize and change instrument operating parameters (or increase the injection volume, if appropriate).

Second Criterion:
Run a set of standards of five or more concentration levels, from 1/2 LOQ, up to the highest concentration level to be included in the analysis. Generate a calibration curve for PFOA and obtain a linear regression with a coefficient of determination (R^2) of at least 0.985 for PFOA. Once this criterion is met, samples may be analyzed with standards interspersed.

4.9. Limit Of Detection (LOD) and Limit Of Quantitation (LOQ)

A Limit of Detection (LOD) and Limit of Quantitation (LOQ) will be reported for each set of samples analyzed. The LOD is defined using the method blank (or reagent blank), and is the concentration (as would be expressed in the original samples) that would correspond to 3 times the peak-to-peak amplitude of the baseline noise in the vicinity of the PFOA peak. If laboratory or instrumental background contamination or interference is present, the LOD will be the concentration that corresponds to three times the amplitude of the background peak at the retention time of PFOA. The LOQ will be defined as 5 times the LOD. PFOA levels in the samples below the LOD will be reported as "Not Detected" (ND). PFOA levels in samples greater than LOD but less than LOQ will be reported as "Not Quantifiable" (NQ). Values at or greater than LOQ will be reported as numerical values, rounded to two significant figures. All raw data will be included in the data package, including peak areas below LOD or LOQ.

LOD/LOQ will be determined, as above, at least quarterly.

4.10. TIME REQUIRED FOR ANALYSIS

A set of 24 samples (6 standards and 18 samples) can be taken through the extraction procedure in approximately 2-3 hours by one person. The LC/MS/MS analysis (standards and 18 samples) will take approximately 7 hours.

5. CALCULATIONS

a. Use Equation 1 to calculate the amount of PFOA found (in ng/L, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program.

Equation 1:

$$\text{PFOA found (ng/L)} = \frac{\text{(Peak area - intercept)}}{\text{slope}} \times \text{DF}$$

DF = factor by which the final volume was diluted, if necessary.

b. For samples fortified with known amounts of PFOA prior to extraction, use Equation 2 to calculate the percent recovery.

Equation 2:

Recovery (%) =

$$\frac{\left[\text{total analyte found (ng/L)} - \text{analyte found in control (ng/L)}\right]}{\text{analyte added (ng/L)}} \times 100$$

Note: Subtract analyte found in control (ng/L) from analyte found (ng/L), if applicable.

c. If necessary, use Equation 3 to calculate the amount of APFO found (in ng/L) using the molecular weight ratio of APFO/PFOA:

Equation 3:

$$\text{APFO found (ng/L)} = \text{PFOA Found} \times \frac{\text{MW APFO (431)}}{\text{MW PFOA (414)}}$$

Note: calculations could also be performed in ppb or ng/mL if concentrations were assigned in ng/mL instead of ng/L when performing the analysis.

For reporting purposes, field samples in which either no peaks or peaks less than the MDL are detected at the corresponding analyte retention time will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention time that are less than the LOQ and greater than or equal to the MDL will be reported as NQ (not quantifiable).

6. SAFETY

The analyst should read the material safety data sheets for all standards and reagents before performing this method. Use universal precautions when handling standards and reagents, including working in fume hoods and wearing laboratory coats, safety glasses, and gloves.

7. REFERENCES

Ellis, D.A.; Martin, J.W.; Muir, D.C.G.; Mabury, S.A. Development of an ^{19}F NMR Method for the Analysis of Fluorinated Acids in Environmental Water Samples. *Anal. Chem.* 2000, *72*, 726-731.

Moody, C.A.; Kwan, W.C.; Martin, J.W.; Muir D.C.G.; Mabury, S.A. Determination of Perfluorinated Surfactants in Surface Water Samples by Two Independent Analytical Techniques: Liquid Chromatography/Tandem Mass Spectrometry and ^{19}F NMR. *Anal. Chem.* 2001, *73*, 2200-2206.

Figure 1. Calibration curve for PFOA

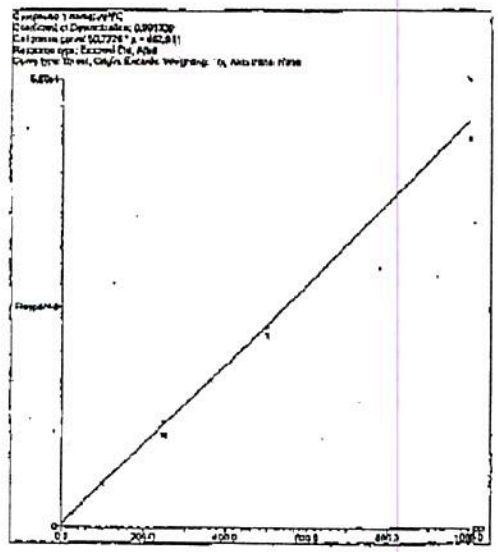

Figure 2. Representative Chromatogram of a 50 ng/L PFOA Standard

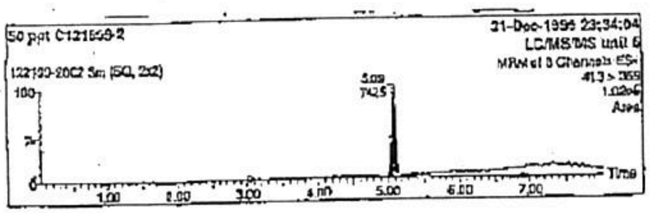

Figure 3. Representative Chromatogram of a 100 ng/L PFOA Standard

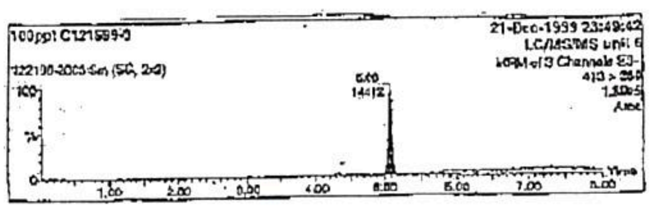

Figure 4. Representative Chromatogram of a 250 ng/L PFOA Standard

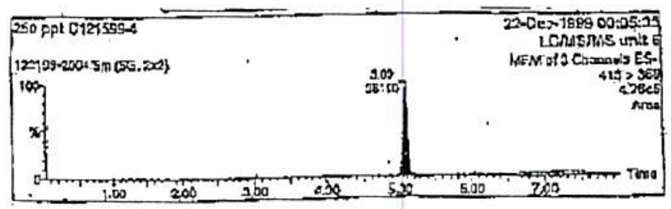

Figure 5. Representative Chromatogram of a 500 ng/L PFOA Standard

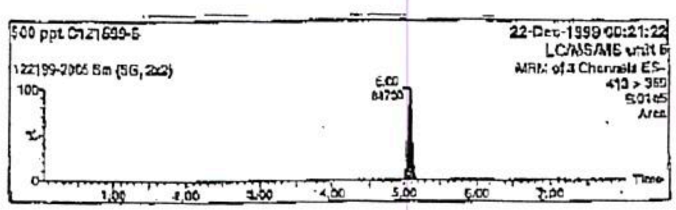

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Figure 6. Representative Chromatogram of 1000 ng/L PFOA Standard

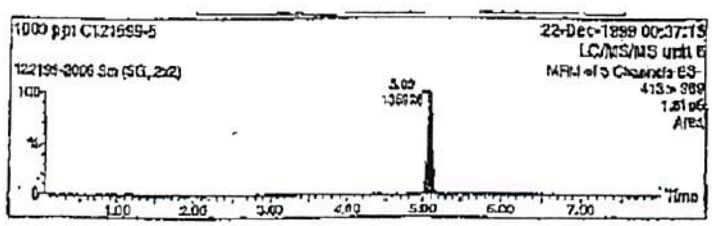

Figure 7. Chromatogram of PFOA in Reagent Control Sample

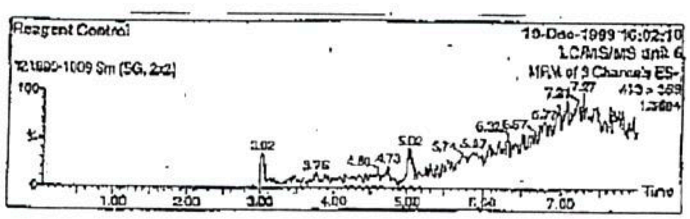

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Figure 8. Chromatogram of PFOA in a Drinking Water Control Sample

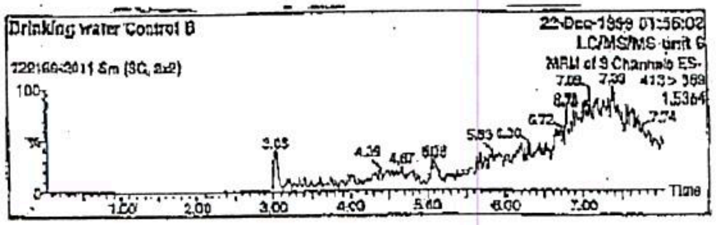

Figure 9. Chromatogram of PFOA in a Drinking Water Control Sample Fortified at 100 ng/L

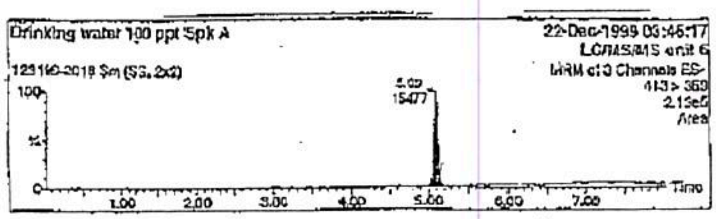

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Figure 10. Chromatogram of PFOA in a Stream Water Control Sample

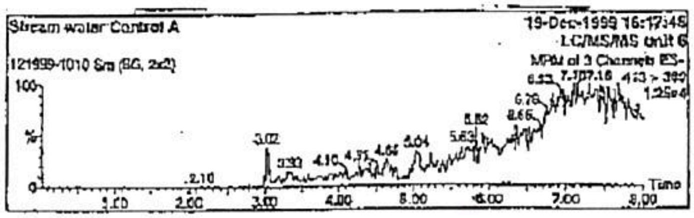

Figure 11. Chromatogram of PFOA in a Stream Water Control Sample Fortified at 100 ng/L

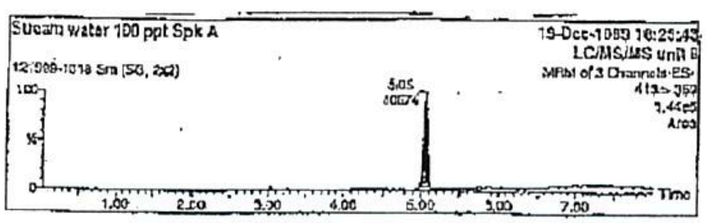

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Figure 12. Chromatogram of PFOA in a Well Water Control Sample

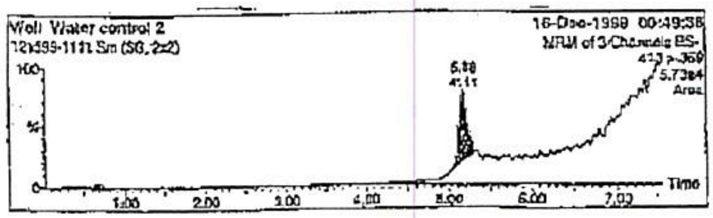

Figure 13. Chromatogram of PFOA in a Well Water Control Sample Fortified at 100 ng/L

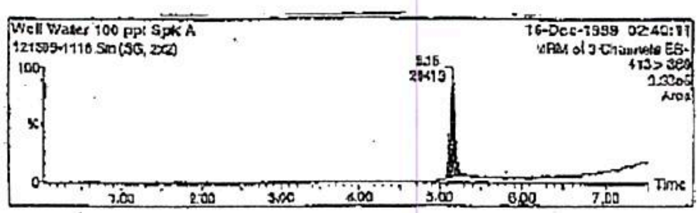

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Figure 14. Chromatogram of PFOA in a Tap Water Control Sample

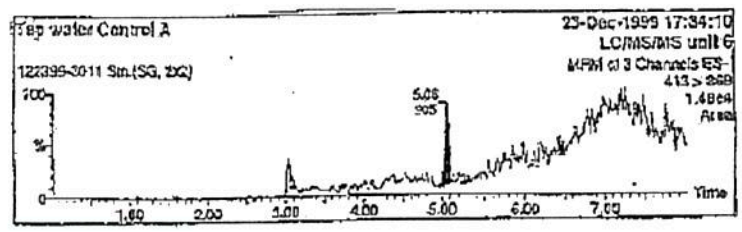
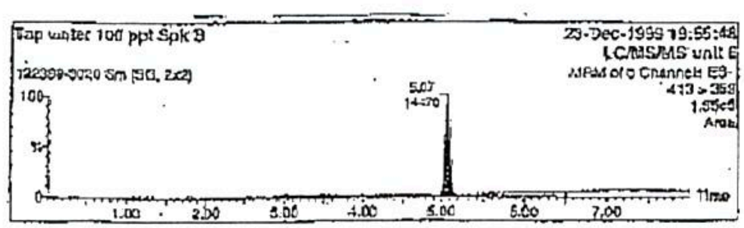


Figure 15. Chromatogram of PFOA in a Tap Water Control Sample Fortified at 100 ng/L



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APPENDIX B

Method of Analysis for the Determination of Perfluorooctanioc Acid (PFOA)
in Filter Paper by LC/MS/MS
Revision 1